Appendix 1

Supporting Experimental Data

As a natural part of the healing process after surgery the body deposits fibrin onto injured tissues. Fibrin acts to seal the injury and allows the deposition of cellular matrix, but may cause tissues that should be separate to adhere to one another and become held together by an adhesion. Adhesions can prevent tissues and organs from moving freely resulting in medical complications requiring treatment.

A series of experiments have been performed to assess the effect of the synthetic lamellar body, LMS-611 on fibrin.

The Effect of the Synthetic Lamellar Body, LMS-611 and Other Phospholipid Mixtures on Fibrin Clot Porosity.

The effect of phosphotidylcholine (PC) (100%), LMS-315 (phosphotidylcholine 65%, sphingomyelin 23% and cholesterol 12%) and LMS-611 (phosphatidylcholine 54%, sphingomyelin 19%, phosphatidylethanolamine 8%, phosphatidylserine 4%, phosphatidylinositol 3% and cholesterol 10%) on the porosity of a fibrin clot were assessed by measuring the volume of saline passing through the clot over a 45 minute period.

By combining fibrinogen and thrombin with the test items (500µl), in a 2ml syringe, an artificial fibrin clot was formed after 60 minutes. A hydrostatic head of saline (0.9% NaCl, 1.5ml) was then added and the flow of saline through the clot collected over a 45 minute period. The saline collected being taken as a measure of the porosity of the clot.

The addition of the synthetic lamellar body, LMS-611 to the fibrin clot was found to increase the porosity of the fibrin clot, as shown by the volume of saline collected after 45 minutes, compared to saline Control, phosphotidylcholine or the phospholipid mixture LMS-315. The latter two test materials were not significantly different from the saline Control (Figure 1).

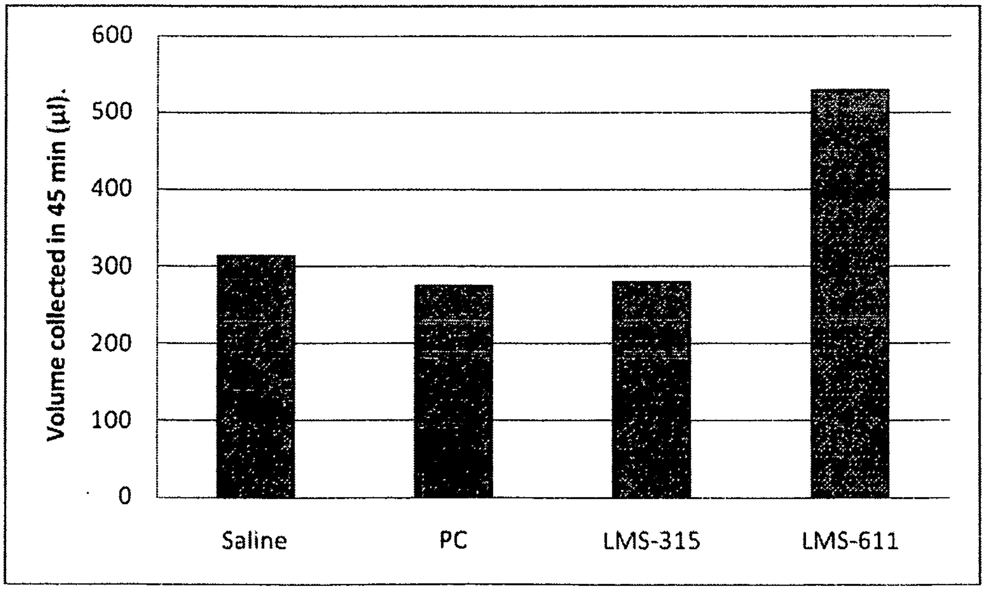

Figure 1. The effect of phosphotidylcholine (PC), LMS-315 and LMS-611 (an example of the claimed composition) on the porosity of a fibrin clot, as measured by the volume of saline penetrating the clot in 45 minutes.

The remaining clots were then visualised using scanning electron microscopy and the clot structures compared. The clot formed in the presence of LMS-611 was found to have a considerably more open structure than those formed in the presence of saline, phosphotidylcholine and LMS-315. The open structure of a fibrin clot formed in the presence of LMS-611 can be seen in the example shown in Figure 2.

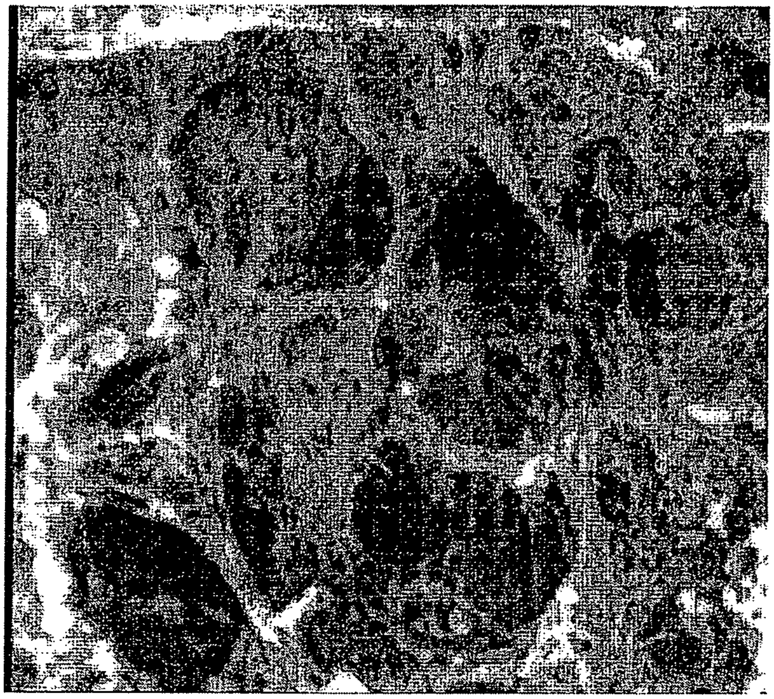

Figure 2. The structure of a fibrin clot treated with LMS-611, as seen using a scanning electron microscope.

As fibrin is the biological gel responsible for adhesions, the administration of the synthetic lamellar body, LMS-611 during surgery will result in permeation and disaggregation of the fibrin and so reduce adhesions.

The Effect of the Synthetic Lamellar Body, LMS-611 and Other Phospholipid Mixtures on Biological Gels *In Vitro.*

Surfactants comprise a mixture of phospholipids and are used to treat infant respiratory distress syndrome. Liposomes are vesicles used as excipients in drug delivery and typically comprise of phospholipids and cholesterol. These phospholipid mixtures act as useful Controls against which to compare the synthetic lamellar body, LMS-611 (phosphatidylcholine, sphingomyelin, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol and cholesterol in amounts as defined previously).

The effect of the surfactants Survanta (25mg/ml) and Curosurf (80mg/ml), and the commercially available, liposomes, L4031 (L-a-phosphatidylcholine, sterylamine and cholesterol) and L4395

(L-a-phosphatidylcholine b-oleoyl-glpalmitoyl, L-a-phosphatidyl-DL-glycerol, dioleoyl and cholesterol) were tested for effect on the adherence of biological gels to an inclined plane. The surfactants had little effect on the adherence of the biological gels to the inclined plane i.e. there was no reduction in the adherence of the biological gel to the inclined plane. Although, Curosurf, on repeated administrations, slowly mobilised DNA which then travelled the distance within the time frame permitted (100% reduction in adherence). The liposomes had a slight effect on the adherence of the gels to the inclined plane. The synthetic lamellar body, LMS-611 (5mg/ml) had an immediate effect, significantly reducing the adherence of **mucus, alginate and DNA** to the inclined plane (Figure 3).

Figure 3. The effect of surfactants, liposomes and LMS-611 on the adherence of different biological gels to an inclined plane.

Methodology: The adherence of biological gels was measured as the resistive flow of the gel down an inclined plane under the influence of gravity (Hubbold, Garner and Chakraborti 2001). Droplets of carboxymethylcellulose (400-800 cps), mucin (5%; from bovine submaxillary glands), DNA (1%; polymeric form from salmon testes) and experimental alginate (manuronic acid) were pipetted in a straight line onto a polystyrene Petri dish, the droplets were tenaciously adherent allowing the dish to be held at a right angle (90°). The droplets were then sprayed with the test article (100µl). The time to travel down the plate was measured. Administration was repeated if the droplet had not travelled the full extent in one minute.